UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF CHEMICAL
SAFETY AND POLLUTION
PREVENTION

July 15, 2013

MEMORANDUM

Subject: Efficacy Review for Per-Ox; EPA Reg. No. 833-4;
DP Barcode: D411121.

From: Marcus Rindal, Microbiologist 7/16/13
Efficacy Evaluation Team
Product Science Branch
Antimicrobials Division (7510P)

Thru: Mark Perry, Team Leader MP
Efficacy Evaluation Team
Product Science Branch
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To: Demson Fuller / Marshall Swindell PM33
Regulatory Management Branch I
Antimicrobials Division (7510P)

Applicant: Alex C. Fergusson, Inc.
5000 Letterkenny Road, Suite 220
Chambersburg, PA 17201

Formulation from the Label:

Active Ingredient	% by wt.
Hydrogen Peroxide	22.00 %
Peroxyacetic Acid	5.25 %
Inert Ingredients:	68.75 %
Total	100.00 %

I. BACKGROUND

The product, Per-Ox (EPA Reg. No. 833-4), is an EPA registered dilutable liquid antimicrobial for use as a industrial/institutional sanitizer only on previously cleaned hard, non-porous food contact surfaces. The label states that Per-Ox is for sanitizing hard, inanimate, non-food contact surfaces and for use in the disinfection of hard, non-porous surfaces in general commercial environments as well as an antimicrobial container rinse to control beverage spoilage microorganisms. The applicant requested to amend the registration of this product to include claims for efficacy against *Listeria monocytogenes* and *Salmonella typhimurium*. The efficacy data were generated at ATS Labs, located at 1285 Corporate Center, Suite 110, Eagan, MN 55121.

The registrant's current submission (DP Barcode: D411121) includes the efficacy evaluations of three batches of the disinfectant (one batch at least 60 days old). This data package also contained a letter dated March 21, 2013 from the applicant, two studies (MRID 490878-01 and 490878-02), a Statement of No Data Confidentiality Claims, EPA Form 8570-1 and the proposed label.

II. USE DIRECTIONS

The proposed product is for industrial/institutional use in dairies, wineries, breweries, beverage plants, meat and poultry processing plants, milk/dairy product processing plants, seafood and produce processing plants, egg processing/packing equipment surfaces, and eating establishments on eating, drinking, and food preparation utensils, countertops and food preparation surfaces, tableware, and plastic, glass, and metal bottles (rinse). The following directions were provided for the preparation and use of the product as described:

Sanitizing of Hard, Non-Porous Food Contact Surfaces: Remove gross particulate matter with warm water flush. Wash equipment with detergent or cleaning solution. Rinse equipment with potable water. Prepare solution by adding 1.0 to 1.7 fluid ounces of product 5 gallons potable water. This provides 90 to 153 ppm peroxyacetic acid and 378 to 644 ppm hydrogen peroxide. Fill closed system with diluted sanitizer solution and allows a contact time of 1 minute. If sanitizing against *Listeria monocytogenes*, use 1.25 to 1.7 fluid ounces of this product to 5 gallons potable water. This will provide 112 to 153 ppm of peroxyacetic acid and 454 to 644 ppm of hydrogen peroxide. For open or not completely closed system, use a coarse spray, mop/wipe or flood technique to apply solution to the surface and allow contact time of 1 minute. Allow surface to drain thoroughly before resuming operation.

General Environmental Surfaces Sanitizing (Non-Food Contact): Wash surface/item with a cleaner or suitable detergent. Rinse surface/item with potable water. Prepare sanitizer solution by adding 1 to 10 fluid ounces of product 15 gallons potable water to prepare a solution containing 30 to 300 ppm of peroxyacetic acid and 126 to 1262 ppm of hydrogen peroxide. Immerse items in diluted sanitizer solution or apply diluted solution using coarse spray, mop/wipe or flood technique and allow contact time for at least 5 minutes. Allow items and/or surfaces to drain adequately or air dry.

Hard Surface Disinfection: Prepare disinfecting solution by adding 3.2 to 30 fluid ounces of the product to 5 gallons of potable water. This will provide 288 to 2700 ppm of peroxyacetic acid and 1211 to 11,340 ppm hydrogen peroxide. Remove gross filth with from surfaces to be disinfected by cleaning with a detergent or suitable cleaning product. Rinse with potable water.

Apply by wiping, mopping, or as a coarse spray. Allow at least 10 minutes contact time, then air dry. Applications on food-contact surfaces require a sterile or potable water rinse following disinfection.

Eating Establishment Sanitizing: Scrape/prewash plates, utensils, cups, glasses, etc. whenever possible. Wash all items with a detergent and rinse thoroughly with potable water. Prepare product solution by adding 1.0 to 1.7 fluid ounces to 5 gallons potable water. This provides 90 to 153 ppm peroxyacetic acid and 378 to 644 ppm hydrogen peroxide. Immerse all items for at least 2 minutes or for a contact time as specified by the local governing sanitation code. If sanitizing against *Listeria monocytogenes,* use 1.2 to 1.7 fluid ounces of this product to 5 gallons potable water. This will provide 112 to 153 ppm of peroxyacetic acid and 454 to 644 ppm of hydrogen peroxide. Place all sanitized items on a rack or drainboard to drain adequately. Air dry if items will not be reused immediately.

Antimicrobial Rinse of Pre-Cleaned or New Returnable or Non-Returnable Containers: To reduce the number of nonpathogenic beverage spoilage organisms such as *Aspergillus versicolor, Byssochlamys fulve, Pediococcus damnosus, Lactobacillus buchneri* and *Saccharomyces cerevisiae.* Prepare solution by adding 7.0 to 30 fluid oz. to 5 gallons of potable water. This will provide 632 to 2,707 ppm of peroxyacetic acid and 2,650 to 11,354 ppm hydrogen peroxide. Apply solution, allowing a minimum contact time of 5 seconds. Allow containers to drain thoroughly, and then rinse with sterile or potable water.

III. AGENCY STANDARDS FOR PROPOSED CLAIMS

Disinfectants for Use on Hard Surfaces (Against a Broad Spectrum of Bacteria): The effectiveness of disinfectants for use on hard surfaces must be substantiated by data derived using the AOAC Use-Dilution Method (for water soluble powders and liquid products) or the AOAC Germicidal Spray Products as Disinfectants Method (for spray products). Sixty carriers must be tested with each of 3 product samples, representing 3 different product lots, one of which is at least 60 days old, against *Salmonella enterica* (ATCC 10708) and *Staphylococcus aureus* (ATCC 6538). To support products labeled as "general disinfectants," killing on 59 out of 60 carriers is required to provide effectiveness at the 95% confidence level.

Sanitizer Test (for inanimate, non-food contact surfaces): The effectiveness of sanitizers for non-food contact surfaces must be supported by data that show that the product will substantially reduce the numbers of test bacteria on a treated surface over those on an untreated control surface. The test surface(s) should represent the type(s) of surfaces recommended for treatment on the label, i.e., porous or non-porous. Products that are represented as "one-step sanitizers" should be tested with an appropriate organic soil load, such as 5 percent serum. Tests should be performed with each of 3 product samples, representing 3 different product lots, one of which is at least 60 days old against *Staphylococcus aureus* (ATCC 6538) and either *Klebsiella pneumoniae* (aberrant, ATCC 4352) or *Enterobacter aerogenes* (ATCC 13048 or 15038). The ASTM method states that the inoculum employed should provide a count of at least 7.5×10^5 colony forming units per carrier. Results must show a bacterial reduction of at least 99.9 percent over the parallel control within 5 minutes.

IV. BRIEF DESCRIPTION OF THE DATA

1. MRID No. 490878-01, "Standard Test Method for Efficacy of Sanitizers Recommended for Inanimate Non-Food Contact Surfaces (Dilutable)," Against *Klebsiella pneumoniae* and *Staphylococcus aureus* with Per-Ox, by Jill Ruhme. Study Completion Date—February 7, 2013. Project Number A14557.

This study was conducted against *Klebsiella pneumoniae* (ATCC 4352) and *Staphylococcus aureus* (ATCC 6538). Three lots (Lot Nos. 5001, 5002, and 5003) of the product, Per-Ox, were tested using ATS Labs protocol no. AFC01011413.NFS (copy provided). Lot 5003 was ≥ 60 days old at the time of testing. The objective of this study was to determine the antimicrobial efficacy of sanitizers on hard, inanimate, non-porous, **non-food contact** surfaces. The test substance was prepared by diluting 0.067 oz/gal defined as 1.0 fl. oz. test substance + 15.0 gal sterilized deionized water. An equivalent dilution of 0.067 oz/gal, defined as 1.0 fl. oz. test substance + 15.0 gal diluent, was prepared using 1.00 mL of the test substance and 1920 mL sterilized deionized water. The prepared test substance was homogenous as determined by visual observation and was used within three hours of preparation. No organic soil load was required. Glass 1" x 1" carriers were dipped in 95% alcohol, rinsed with deionized water, and air dried before sterilization. The carriers were placed into a vessel and sterilized in a hot air oven for ≥2 hours at ≥180°C. After sterilization, each carrier was placed into a sterile Petri dish. The test organisms were prepared from a stock slant, an initial tube (10 mL) of culture broth was inoculated. This culture was termed the "initial broth suspension." From this initial broth suspension, a minimum of three daily transfers using 1 loopful (10 µL) of culture into 10 mL of culture media was performed on consecutive days prior to use in testing procedure. The appropriate growth medium was subcultured using a daily transfer (more than 3, but less than 30 transfers) for each test organism. A 48-54 hour culture was vortex-mixed and allowed to settle for ≥15 minutes. The upper 2/3rds of the culture was removed and transferred to a sterile vessel for use in testing. The culture was thoroughly mixed prior to use. Sterile carriers were inoculated with 0.02 mL (20.0 µL) of culture using a calibrated pipettor spreading the inoculum to within approximately 1/8 inch of the edges of the carrier. The inoculated carriers were dried for 37 minutes at 35-37°C and 40-40.1% relative humidity with the Petri dish lids slightly ajar for *Staphylococcus aureus* and intact for *Klebsiella pneumoniae*. Following the completion of drying, each of the five test carriers was transferred to individual sterile jars using sterile forceps. Carriers were placed inoculated-side down within the jars. Using staggered intervals, 5.0 mL of prepared test substance was transferred to each jar. The liquid completely covered the carrier during exposure. The remaining test carriers were treated using staggered intervals. The carriers were allowed to expose at room temperature (20°C) and 19% relative humidity for 5 minutes. Following exposure, 20.0 mL of neutralizer was transferred to the jars using identical staggered intervals. The carriers were vortex-mixed. For *Staphylococcus aureus*, within 30 minutes of neutralization, duplicate 1.00 mL aliquots of the neutralized solution (10^0) and duplicate 1.00 mL aliquots of a ten-fold serial dilution
(10^{-1}) were plated onto the recovery agar plate medium. For *Klebsiella pneumoniae*, within
30 minutes of neutralization, duplicate 1.00 and 0.100 mL aliquots of the neutralized solution (10^0) were plated onto the recovery agar plate medium. The plates were incubated at 35-37°C for 48±4 hours. Following incubation, the subcultures were visually enumerated. Controls included those for numbers, purity, sterility, and neutralization confirmation.

2. MRID No. 490878-02, "AOAC Use-Dilution Method," Against *Salmonella enterica* and *Staphylococcus aureus* with Per-Ox, by Becky Lien. Study Completion Date—January 15, 2013. Project Number A14494.

This study was conducted against *Salmonella enterica* (ATCC 10708) and *Staphylococcus aureus* (ATCC 6538). Three lots (Lot Nos. 5001, 5002, and 5003) of the product, Per-Ox, were tested using ATS Labs protocol no. AFC01121312.UD (copy provided). Lot 5003 was ≥ 60 days old at the time of testing. The purpose of the study was to determine the effectiveness of the sponsor's product as a disinfectant for use on hard surfaces against a broad spectrum of bacteria. The test substance was prepared by diluting 0.64 oz/gal defined as 3.2 fl. oz of test substance + 5.0 gallons sterilized deionized water. An equivalent dilution of 0.64 oz/gal, defined as 3.2 fl. oz of test substance + 5.0 gallons diluent, was prepared using 4.0 mL of the test substance and 800 mL sterilized deionized water. The prepared test substance was homogenous as determined by visual observation and was used within three hours of preparation. Ten (10.0) mL aliquots of the test substance at the concentration under test were transferred to sterile 25 x 150 mm tubes, placed in a 20±1°C (20.0°C) water bath and allowed to equilibrate for ≥10 minutes prior to testing. For preparation of the test organisms, a 10 µL aliquot of a thawed cryovial of stock organism broth culture was transferred to an initial 10 mL tube of growth medium. The tube was mixed and the initial culture was incubated for 24±2 hours at 35-37°C. Following incubation and without vortex mixing the initial culture, a 10 µL aliquot of culture was transferred to sufficient 10 mL tubes of culture medium (daily transfer #1). The final test culture was incubated for 48-54 hours at 35-37°C. Each test culture was vortex mixed for 3 to 4 seconds and allowed to stand for ≥10 minutes prior to use. After this time, the upper portion of the culture was removed, leaving behind any clumps or debris and was pooled in a sterile vessel. The culture was diluted using sterile growth medium by combining 100.0 mL of test organism suspension with 100.0 mL of sterile growth medium. The final test culture was mixed thoroughly prior to use. The culture was transferred to the penicylinders and the carriers were immersed for 15±2 minutes in a prepared suspension at a ratio of one carrier per one mL of culture. A maximum of 100 carriers were inoculated per vessel. The inoculated carriers were transferred to sterile Petri dishes matted with filter paper. No more than twelve carriers were placed in each Petri dish. The carriers were dried for 38 minutes at 35-37°C at a 40% relative humidity. Each contaminated and dried carrier was placed into a separate tube containing 10.0 mL of the test substance at its use-dilution. Immediately after placing each test carrier in the test tube, the tube was swirled using approximately 2-3 gentle rotations to release any air bubbles trapped in or on the carrier. The carriers were exposed for 10 minutes at 20.0°C. Following the 10 min exposure time, each medicated carrier was transferred by wire hook at staggered intervals to 10 mL of neutralizing subculture medium. To accomplish this, the carrier was removed from the disinfectant tube with a sterile hook, tapped against the interior sides of the tube to remove the excess disinfectant and transferred into the subculture tube. Tapping the carrier against the approximate upper third of the tube was avoided. All subculture vessels and control plates were incubated for 48±2 hours at 35-37°C. Following incubation, the subcultures were visually examined for the presence or absence of growth. Representative subculture tubes showing growth were subcultured, stained and/or biochemically assayed to confirm or rule out the presence of the test organism. Controls included those for numbers, purity, viability, sterility (carriers and media), and neutralization confirmation.

V. RESULTS

Table 1.
MRID 490878-01 Efficacy Results for Sanitizer, Non-Food Contact Surface Test

Sanitizer Test Results Summary					
Test Substance	Test Organism	Carrier #	Survivors		Percent Reduction
			1.00 mL plated of 10^0 dilution	0.10 mL plated of 10^0 dilution	
Batch 5001	*Klebsiella pneumoniae* (ATCC 4352)	1	0,0	0,0	>99.9%
		2	0,0	0,0	
		3	0,0	0,0	
		4	0,0	0,0	
		5	0,0	0,0	
Batch 5002		1	0,0	0,0	>99.9%
		2	0,0	0,0	
		3	0,0	0,0	
		4	0,0	0,0	
		5	0,0	0,0	
Batch 5003 (≥60 days old)		1	0,0	0,0	>99.9%
		2	0,0	0,0	
		3	0,0	0,0	
		4	0,0	0,0	
		5	0,0	0,0	
K. pneumoniae Carrier Population Control Results			Average Log	7.32	
			Geometric Mean (CFU/carrier)	2.09×10^7	
Test Substance	Test Organism	Carrier #	Survivors		Percent Reduction
			1.00 mL plated of 10^0 dilution	1.00 mL plated of 10^{-1} dilution	
Batch 5001	*Staphylococcus aureus* (ATCC 6538)	1	0,0	0,0	>99.9%
		2	0,0	0,0	
		3	0,0	0,0	
		4	0,0	0,0	
		5	0,0	0,0	
Batch 5002		1	0,0	0,0	>99.9%
		2	0,0	0,0	
		3	0,0	0,0	
		4	0,0	0,0	
		5	0,0	0,0	
Batch 5003 (≥60 days old)		1	0,0	0,0	>99.9%
		2	0,0	0,0	
		3	0,0	0,0	
		4	0,0	0,0	
		5	0,0	0,0	
S. aureus Carrier Population Control Results			Average Log	6.32	
			Geometric Mean (CFU/carrier)	2.09×10^6	

Table 2.
MRID 490878-02 Efficacy Results for Broad Spectrum Disinfectant Test – AOAC UDM

Use-Dilution Test Results Summary				
Test Substance	Test Organism	Sample Dilution	Number of Carriers	
			Exposed	Showing Growth
Batch 5001	*Salmonella enterica* (ATCC 10708)	0.64 oz/gal	60	0
	Staphylococcus aureus (ATCC 6538)		60	0
Batch 5002	*Salmonella enterica* (ATCC 10708)		60	0
	Staphylococcus aureus (ATCC 6538)		60	0
Batch 5003 (≥60 days old)	*Salmonella enterica* (ATCC 10708)		60	1
	Staphylococcus aureus (ATCC 6538)		60	0
S. aureus Carrier Population Control Results			Average Log_{10}	6.84
Salmonella enterica Carrier Population Control Results			Average Log_{10}	5.51

VI. CONCLUSION

1. The submitted efficacy data (MRID No. 490878-01) supports the use of the product, Per-Ox, as non-food contact sanitizer against *Staphylococcus aureus* and *Klebsiella pneumoniae* for a contact time of 5 minutes when prepared 0.067 fl. oz/gallon, on previously cleaned surface (see Table 1). The carrier population controls for both organisms tested exceeded the ASTM stated minimum of at least 7.5 x 10^5 colony forming units per carrier. Results for both organisms tested demonstrated a bacterial reduction of at least 99.9 percent over the parallel control within 5 minutes. Controls were acceptable as reported.

2. The submitted efficacy data (MRID No. 490878-02) supports the use of the product, Per-Ox, as a broad spectrum disinfectant against *Staphylococcus aureus* and *Salmonella enterica* for a contact time of 10 minutes when prepared 0.64 fl. oz/gallon, on previously cleaned surface (see Table 2). Killing on at least 59 out of 60 carriers tested was achieved for both organisms tested against all three lots. Controls were acceptable as reported.

VII. LABEL

1. The proposed label claim is acceptable regarding the use of the product, Per-Ox, as a non-food contact sanitizer against *Staphylococcus aureus* and *Klebsiella pneumoniae,* at a use rate of 1.0 to 10 fl. oz/ 15 gallons of potable water, for a contact time of 5 minutes. **Acceptable efficacy data was provided to support the proposed claim**.

2. The proposed label claim is acceptable regarding the use of the product, Per-Ox, as a broad spectrum disinfectant against *Staphylococcus aureus* and *Salmonella enterica,* at a use rate of 3.2 to 30 fl. oz/ 5 gallons of potable water, for a contact time of 10 minutes. **Acceptable efficacy data was provided to support the proposed claim**.

3. Page 5-6, in the panel with use directions, the directions for Sanitizing Tableware, Final Sanitizing Bottle Rinse, and Sanitizing of Hatching Eggs, no contact time is stated. Indicate contact times supported by the submitted efficacy data.